Example 12: BAP-1 WT protein, protease mutants, siRNA and antisense functional hit are antiproliferative.

The BAP-1 functional hit identified in the retroviral screen is in the antisense orientation. (Figure 2). Expression of the functional hit in a tumor cell line, *e.g.*, A549 cells, or in untransformed cells, *e.g.*, HMEC or PrEc cells, was antiproliferative. (See, *e.g.*, Figures 3, and 34-35.)

Dominant negative mutants of BAP-1 were made by mutating residues in the protease domain. (See, *e.g.*, Figure 29.) Using two different assays, expression of BAP-1 wild-type and protease mutants was antiproliferative in tumor cell lines, *i.e.*, HeLa cells and H1299 cells. (See, *e.g.*, Figures 30-33). siRNA molecules derived from the BAP-1 nucleic aicd were shown to be antiproiferative in HeLa cells and H1299 cells. (See, *e.g.*, Figures 36-37.)

Example 13: BAP-1 is a ubiquitin protease.

GST-Bap-1 was expressed in and purified from SF9 cells. (See, *e.g.*, Figures 38-39.) Using a fluorogenic ubiuquiting cleavage assay, BAP-1 was shown to be an active ubiquitin protease, with a Km of 0.5 μM for the substrate UbAMC. (See, *e.g.*, Figures 40-42.) UbCHO was also demonstrated to be a specific inhibitor of BAP-1. (See, *e.g.*, Figure 43.)

Assays for ubiquitin hydrolase activity (*e.g.*, to assay BAP-1 activity) can also be performed as described in U.S. Patent No. 6,307,035 and Mayer and Wilkinson, *Biochemistry* 28:166(1989) using the glycine 76 ethyl ester of ubiquitin as a substrate. Peak areas can be integrated and normalized with respect to the ubiquitin standard.

Example 14: NP95 WT protein, ring finger mutants, siRNA and functional hit are antiproliferative.

The NP95 (G1-2635) functional hit (G1-2635) identified in the retroviral screen is in the sense orientation. (Figure 2). Expression of the functional hit in a tumor cell line, *e.g.*, A549 cells, or in untransformed cells, *e.g.*, HMEC or PrEc cells, was antiproliferative. (See, *e.g.*, Figures 6, and 44-45.) siRNA molecules derived from the NP-95 nucleic acid were shown to be antiproiferative in PrEc and HUVEC cells and H1299 cells. (See, *e.g.*, Figures 46-47, and 57.)

Using real time PCR analysis, NP95 mRNA expression was shown to be overexpressed in tumor tissue relative to normal tissue from the same patient. Increased

Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to persons of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. *See, e.g.*, Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen *et al., J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al., Science*, 251:767-777 (1991); Sheldon *et al., Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al., Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

IMMUNOLOGICAL DETECTION OF BAP-1, NP95, FANCA, DDX9, IGF1R, UBE2V1, ALDEHYDE DEHYDROGENASE, PYRUVATE KINASE, G6PD, HCDR-3, DDX21, ARK2, TRANSMEMBRANE 4 SUPERFAMILY MEMBER 1, OR ERCC1 POLYPEPTIDES

In addition to the detection of BAP-1, NP95, FANCA, DDX9, IGF1R, UBE2V1, aldehyde dehydrogenase, pyruvate kinase, G6PD, HCDR-3, DDX21, ARK2,

Imamura, *et al., Nuc. Acids Res.* 26(9):2063 (1998); and Zhang *et al., J. Cell. Sci.* 112:2693 (1999)). Vectors containing DNA encoding DDX9 complement yeast that have mutations in *prp8-1*, the yeast homolog of DDX9 (*see* Imamura *et al.*). Helicase assays known to those of skill in the art can be used, e.g., to identify modulators of DDX9.

IGF1R encodes a cell surface tyrosine kinase receptor and binds to IGF1 ligand (*see, e.g.*, Nakae *et al., Endocr. Rev.* 22(6):818 (2001); Flier *et al., Proc. Nat'l Acad. Sci. USA* 83:664-668 (1086); Francke *et al., Cold Spring Harb. Symp. Quant. Biol.* 51(Pt. 2):855-866 (1986); Ullrich *et al., EMBO J.* 5:2503-2512 (1986); Cooke *et al., Biochem. Biophys. Res. Commun.* 177:1113-1120 (1991); Abbott *et al., J. Biol. Chem.* 267:10759-10763 (1992); Werner *et al., Proc. Nat'l Acad. Sci. USA* 93:318-8323 (1996); Grant *et al., J. Clin. Endocrinol. Metab.* 83:3252-3257 (1998); and Butler & LeRoith, *Endocrinology* 142(5):1685 (2001)). Upon ligand binding, the receptor undergoes a conformational change which enables it to bind ATP, thereby increasing their kinase activity and modulate cell proliferation (*see* Nakae *et al.*). IGF1R deficient mice develop cell proliferation disorders, including muscle hypoplasia due to decreased cell numbers; IGF1R null mice develop cell proliferation disorders including dwarfism (*Id.*). Overexpression of IGF1R has been linked to increased radioresistance of breast cancer cells (*see* Macaulay *et al., Oncogene* 22(6):4029 (2001)). Ligand binding assays, autophosphorylation assays, kinase assays, and signal transduction assays known to those of skill in the art can be used, e.g., to identify modulators of IGF1R.

UBE2V1 encodes a protein that has been show to play a role in cell cycle regulation (*see, e.g.*, Rothofsky *et al., Gene* 195:141-149 (1997); Sancho *et al., Mol. Cell. Biol.* 18:576-589 (1998); Ma *et al., Oncogene* 17:1321-1326 (1998); Hofmann & Pickart, *Cell* 96:645-653 (1999); Deng *et al., Cell* 103:351-361 (2000); and Thomson *et al., Genome Res.* 10:1743-1756 (2000)). Constitutive expression of exogenous UBE2V1 inhibits the capacity of colorectal adenocarcinoma cells to differentiate upon confluence and inhibits the mitotic kinase cdk1, thereby inducing the cells to arrest at the G_2-M phase of the cell cycle (*see,* Sancho *et al., Mol. Cell. Biol.* 18(1):576 (1998) and Stubbs *et al., Am. J. Path.* 154(5):1335 (1999)). UBE2V1 has four alternatively spliced transcripts that encode proteins with the conserved Ubc domain of E2 enzymes and unique N-terminal sequence (see Figure 21). Ubiquitination assays, e.g., ubiquitin ligase assays, known to those of skill in the art, can be used to identify modulators of UBE2V1.

Aldehyde dehydrogenases form a superfamily of NADP+ dependent enzymes that are involved in several distinct metabolic pathways (*see* Vasilou *et al., Chem. Biol.*

fluorescence analysis of IGF1R infected A549.tTA cells. Figure 24 lower right illustrates cell tracker assay date from IGF1R infected A549.tTA cells.

Figure 25 provides an illustration of the relevant domains of UBE2V1, including the ubiquitin conjugating enzyme domain.

Figure 26 illustrates cell tracker assay data demonstrating that GFP-fused UBE2V1 is antiproliferative in A549 cancer cells. The UBE2V1 construct is the functional hit isolated in the retroviral screen. Figure 26 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 26 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 26 lower left illustrates fluorescence analysis of UBE2V1 infected A549.tTA cells. Figure 26 top right illustrates cell tracker assay date from UBE2V1 infected A549.tTA cells.

Figure 27 shows four alternatively spliced UBE2V1 transcripts.

Figure 28 provides some cDNA sequence isolated from a cell tracker assay for cDNAs that regulate the cell cycle, *i.e.,* functional hits from the retroviral screen.

Figure 29 provides dominant negative mutants of BAP-1. Mutated residues are shown with arrows.

Figure 30 provides evidence that expression of Bap1 WT and protease mutants is antiproliferative in HeLa cells.

Figure 31 provides evidence tthat expression of Bap1 WT protein is antiproliferative in HeLa cells in the Celltracker assay.

Figure 32 provides evidence that expression of Bap1 protease mutants is slightly more antiproliferative than expression of Bap1 WT in H1299 cells.

Figure 33 provides evidence expression of Bap1 WT and Bap1 protease mutants is antiproliferative in H1299 cells in the Celltracker assay.

Figure 34 provides evidence that the Bap1 functional hit G32D8 is antiproliferative in HMEC cells.

Figure 35 provides evidence that the Bap1 functional hit G3-2D8 is antiproliferative in PrEC cells.

Figure 36 provides evidence that BAP1 specific siRNA has an antiproliferative effect on HeLa cells.

Figure 37 provides evidence that BAP1 specific siRNA induces G1 arrest in H1299 cells.

Figure 38 provides evidence that soluble GST-Bap1 protein can be expressed from SF9 cells. GST-Bap1 was produced using the baculovirus transfer vector pDEST20

Figure 16 provides a nucleotide (SEQ ID NO:23) and an amino acid (SEQ ID NO:24) sequence of human ARK2.

Figure 17 provides a nucleotide (SEQ ID NO:25) and an amino acid (SEQ ID NO:26) sequence of human transmembrane 4 superfamily member 1.

Figure 18 provides a nucleotide (SEQ ID NO:27) and an amino acid (SEQ ID NO:28) sequence of human ERCC1.

Figure 19 provides an illustration of certain relevant domains of FANCA, including the aldehyde dehydrogenase cysteine active site, FKBP-type peptidyl-prolyl cis-trans isomerase signature 1 site, the PX site, and the peptidase S8 site.

Figure 20 illustrates cell tracker assay data demonstrating that GFP-fused FANCA is antiproliferative in A549 cancer cells. The FANCA construct is the functional hit isolated in the retroviral screen. Figure 20 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 20 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 20 lower left illustrates fluorescence analysis of FANCA infected A549.tTA cells. Figure 20 lower right illustrates cell tracker assay date from FANCA infected A549.tTA cells.

Figure 21 provides an illustration of certain relevant domains of DDX9, including the double stranded RNA binding motif, the DEAD/DEAH box helicase domain, the helicase conserved C terminal domain, and the GLN3 protein domain.

Figure 22 illustrates cell tracker assay data demonstrating that GFP-fused DDX9 is antiproliferative in A549 cancer cells. The DDX9 construct is the functional hit isolated in the retroviral screen. Figure 22 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 22 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 22 lower left illustrates fluorescence analysis of DDX9 infected A549.tTA cells. Figure 22 lower right illustrates cell tracker assay date from DDX9 infected A549.tTA cells.

Figure 23 provides an illustration of certain relevant domains of IGF1R, including the receptor L domain, the furin-like cysteine rich region, the fibronectin type II domain, the transmembrane domain, and the kinase domain.

Figure 24 illustrates cell tracker assay data demonstrating that GFP-fused IGF1R is antiproliferative in A549. The IGF1R construct is the functional hit isolated in the retroviral screen. Figure 24 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 24 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 24lower left illustrates

data from GFP infected A549.tTA control cells. Figure 3 lower left illustrates fluorescence analysis of BAP-1 infected A549.tTA cells. Figure 3 lower right illustrates cell tracker assay date from BAP-1 infected A549.tTA cells.

Figure 4 provides a nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of human NP95.

Figure 5 provides an illustration of the relevant domains of NP95, including the ubiquitin like domain, the zinc finger domain, the nuclear protein domain, and the ubiquitin ligase domain.

Figure 6 illustrates cell tracker assay data demonstrating that GFP-fused NP95 is antiproliferative in A549. The NP-95 construct is the functional hit isolated in the retroviral screen. Figure 6 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 6 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 6 lower left illustrates fluorescence analysis of NP95 infected A549.tTA cells. Figure 6 lower right illustrates cell tracker assay date from NP95 infected A549.tTA cells.

Figure 7 provides a nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequence of human FANCA.

Figure 8 provides a nucleotide (SEQ ID NO:7) and an amino acid (SEQ ID NO:8) sequence of human DDX9.

Figure 9 provides a nucleotide (SEQ ID NO:9) and an amino acid (SEQ ID NO:10) sequence of human IGF1R.

Figure 10 provides a nucleotide (SEQ ID NO:11) and an amino acid (SEQ ID NO:12) sequence of human UBE2V1.

Figure 11 provides a nucleotide (SEQ ID NO:13) and an amino acid (SEQ ID NO:14) sequence of human aldehyde dehydrogenase.

Figure 12 provides a nucleotide (SEQ ID NO:15) and an amino acid (SEQ ID NO:16) sequence of human pyruvate kinase.

Figure 13 provides a nucleotide (SEQ ID NO:17) and an amino acid (SEQ ID NO:18) sequence of human G6PD.

Figure 14 provides a nucleotide (SEQ ID NO:19) and an amino acid (SEQ ID NO:20) sequence of human HCDR-3.

Figure 15 provides a nucleotide (SEQ ID NO:21) and an amino acid (SEQ ID NO:22) sequence of human DDX21.

threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

A further embodiment of the invention provides a method of modulating cell cycle arrest in a subject. A therapeutically effective amount of a BRCA-1-Associated Protein-1 (BAP-1), Nuclear Protein 95 (NP95), Fanconi anemia group A protein (FANCA), DEAD/H box polypeptide 9 (DDX9), insulin-like growth factor 1 receptor (IGF1R), ubiquitin-conjugating enzyme E2 variant 1 (UBE2V1), aldehyde dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase, HCDR-3, DEAD/H box polypeptide 21 (DDX21), serine threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide is administered to the subject. The BRCA-1-Associated Protein-1 (BAP-1), Nuclear Protein 95 (NP95), Fanconi anemia group A protein (FANCA), DEAD/H box polypeptide 9 (DDX9), insulin-like growth factor 1 receptor (IGF1R), ubiquitin-conjugating enzyme E2 variant 1 (UBE2V1), aldehyde dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase, HCDR-3, DEAD/H box polypeptide 21 (DDX21), serine threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

Other embodiments and advantages of the present invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a nucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of human BAP-1.

Figure 2 provides an illustration of the relevant domains of BAP-1, including the ubiquitin hydrolase domain and the DNA binding domain. Also shown is the BAP-1 functional hit (G3-2D8) isolated in the retroviral screen. The functional hit is in the antisense orientation.

Figure 3 illustrates cell tracker assay data demonstrating that GFP-fused BAP-1 is antiproliferative in A549 cells. The BAP-1 construct is the functional hit isolated in the retroviral screen. Figure 3 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 3 top right illustrates cell tracker assay